1.0 Purpose and Scope:

1.1 This procedure describes the analysis of samples by headspace gas chromatography for ethyl alcohol and related volatile organic compounds.

1.2 Samples include whole blood which is thought to contain alcohol.

1.3 The scope of this SOP includes autosampler, chromatograph and software set-up.

2.0 Responsibility:

2.1 All analysts having the responsibility for analysis of blood or other samples for alcohol content are responsible for following this procedure.

2.2 This procedure is reviewed periodically by Alcohol Program staff. Necessary revisions are made at that time or when there is an identified need to change this written procedure to be compatible with changing needs in the analytical process.

2.3 All analysts performing this procedure for the purpose of reporting analytical results for forensic purposes must be fully trained and demonstrate initial proficiency in the use of this procedure. All analysts must show ongoing proficiency by successfully analyzing at least one PT sample annually.

3.0 Precautions and Safety Directives:

3.1 Prepared samples can be held at room temperature before analysis is begun for a maximum of 48 hours after preparation. Samples are typically analyzed within 24 hours of preparation.

4.0 Procedure

4.1 Principle of Measurement

4.1.1 Alcohol and related volatile organic compounds are determined in blood by Headspace Gas Chromatography with Flame Ionization Detection. The sample, a mixture of an internal standard solution, a surrogate and the sample to be analyzed, is heated in a vial sealed with a septum. This is allowed to equilibrate so that equal amounts of the volatile compound are present in the liquid and headspace. A portion of the vapor above the heated sample is transferred to a sample loop and injected onto the column of the gas chromatograph.

4.1.2 In the flame ionization detector (FID), the vaporized alcohol or other volatile compound mixed with hydrogen enters a jet where it is burned in an air atmosphere. The jet itself serves as one electrode and a second electrode is placed above the flame. A potential is applied across these electrodes. When molecules enter the flame, ionization occurs yielding a current flow which after proper

amplification, may be displayed on the computer terminal. The FID is a mass-sensitive detector and its response is proportional to the total number of ions entering the detector per unit time, independent of its concentration in the gas.

4.2 Equipment & Materials

4.2.1 All equipment and materials are located in room 265 unless otherwise stated.

4.2.2 Agilent Technologies 6890N Gas Chromatograph with flame ionization detector.

4.2.3 Teledyne-Tekmar HT3 Headspace Autosampler.

4.2.4 Desktop PC and printer with ChromPerfect Spirit Chromatography and HT3 TekLink Software Packages.

4.2.5 Compressed Hydrogen-UHP Grade.

4.2.6 Compressed Helium-UHP Grade.

4.2.7 Compressed Air.

4.3 Sample and Control Preparation

4.3.1 Prepare calibration, control and samples as described in the Sample Preparation for Alcohol Analysis by Headspace GC-FID (P-ALC 101).

4.4 Data System Setup

4.4.1 Set up the ChromPerfect Spirit Chromatography data system as described in Appendix A.

4.4.2 Upon completion of a passing calibration curve, save a copy of the calibration file.

4.5 Chromatograph Setup

4.5.1 Assure that the Helium carrier gas is turned on with an appropriate delivery pressure (approximately 45 psi) and that the amount remaining in the supply cylinder is at 500 psi or greater. If not, replace the tank.

4.5.2 Assure that the Air tank is turned on with an appropriate delivery pressure (approximately 45 psi) and that the remaining cylinder pressure is at 200 psi or greater. If not, replace the tank.

4.5.3 Assure that the Hydrogen fuel tank is turned on with an appropriate delivery pressure (approximately 18 psi) and that the remaining cylinder pressure is at 200 psi or greater. If not, replace the tank.

4.5.4 Ensure that the FID is flame is lit and the GC status is "ready".

4.5.5 Review all operating parameters and adjust as necessary to assure they agree with the settings listed in Appendix C.

4.6 **Autosampler Setup**

4.6.1 Review all operating parameters and adjust as necessary to assure they agree with the settings listed in Appendix B.

5.0 Emergency or High Priority Situations

5.1 The Commissioner of Public Safety, Laboratory Director or Alcohol Program Supervisor can designate samples as high priority.

5.2 High priority samples are analyzed as soon as possible after successful calibration.

5.3 Priority sample results are reviewed and released as soon as they are available, once they pass the quality assurance criteria.

6.0 Quality Control and Corrective Action

6.1 All analytical sequences must contain:

6.1.1 An aqueous blank sample.

6.1.2 A Timing Mix sample containing: Acetaldehyde, Methanol, Ethanol, Isopropanol and Acetone.

6.1.3 Five calibration standards having known alcohol concentrations expressed to three decimal places of the following concentrations:

6.1.3.1 STDA ~0.005%
6.1.3.2 STD B ~0.020%
6.1.3.3 STD C ~0.080%
6.1.3.4 STD D ~0.200%
6.1.3.5 STD E ~0.400%

6.1.4 Duplicate samples of a level one (1) whole blood control obtained from Cliniqa Controls or equivalent NIST traceable control. The mean of the two replicates must be within 10% of the certified value.

6.1.5 A calibration check sample using STD C, which is analyzed in duplicate after every 10^{th} sample and at the end of every run. The mean of the two replicates must be within 10% of the known value. If not, then all analyses from the last acceptable CCS must be repeated. If one replicate fails due to a preparation error, it may be dropped and the remaining replicate may be used as the check sample.

6.1.6 All samples will be run in duplicate. The two sample analyses must be within 10% of the mean result for that sample. If not then the analysis must be repeated (two new preparations from the sample) and the two new replicates must be within 10% of the mean result for that sample. The number of samples used to report the average will be determined on a case by case basis.

6.1.7 Calibration standards must be within 10% of their known values except STD A which must be within 20% of its known value.

6.1.8 The correlation coefficient of the calibration line must be 0.99 or greater and the average error must not be greater than 10%. If not, the calibration must be repeated.

6.1.9 A calibration curve will be run each day samples are analyzed. Additional samples may be analyzed on a calibration provided that a set of calibration check samples are analyzed before and after samples.

7.0 Preventive Maintenance and Backup

7.1 **Agilent 6890N Gas Chromatograph**:

7.1.1 Refer to the Agilent 6890N Gas Chromatograph Reference Manual located in room 265 for repair and maintenance information.

7.2 **Teledyne-Tekmar HT3 Headspace Autosampler**

7.2.1 Refer to the Teledyne-Tekmar HT3 Headspace Autosampler Users Guide located in room 265 or the electronic users guide found in the TekLink software for repair and maintenance information.

7.3 **Backup**

7.3.1 If the laboratory lacks analytical ability for greater than 10 business days, samples will be sent to a qualified reference lab for analysis.

8.0 References and Appendices

8.1 Sample Preparation for Alcohol Analysis by Headspace GC-FID (P-ALC 101).

8.2 Alcohol Analysis Data Review and Reporting (P-ALC 103).

8.3 Agilent 6890N Gas Chromatograph Reference Manual.

8.4 Teledyne-Tekmar HT3 Headspace Autosampler Users Guide.

8.5 ChromPerfect User Manual.

8.6 Appendix A, ChromPerfect Spirit Software Setup.

8.7 Appendix B, Teledyne-Tekmar HT3 Autosampler Setup.

8.8 Appendix C, Parameter Settings for 6890N Gas Chromatograph.

Appendix A

ChromPerfect Spirit Software Setup

1. In Microsoft Explorer, go to C: Alcohol Data and create a new folder. Name it VFLMMDDYYYY where MM is the two-digit month, DD is the two-digit day and YYYY is the four-digit year.

2. Go to ChromPerfect File Editor (yellow icon) and click on "File" in the upper left corner, drag menu down to "New." Select "Sequence" as the file type and load an empty file.

3. In the raw file base name, type C:\Alcohol Data\VFLMMDDYYYY\VFLMMDDYYYY. This establishes the path name for the new files. The first VFLMMDDYYYY is the folder you made in Explorer; the second VFLMMDDYYYY is the raw file base name.

4. Put a 1 in the space for cycle number.

5. Fill in the sample name. This will usually be "Blank" for a new run.

6. Click on the […] box to the right of the method file space. Click on the down arrow and select the appropriate method.

7. At this point, it's prudent to check that everything is ok by clicking on the "Validate" button at the lower right of the screen. If ok, proceed. If not, fix the problem (hints will be given in red on the screen.)

8. On the bottom left use the "Insert Entry" button to add extra entries to the sequence. Change the names after all of the spaces needed are established.

9. Click on the cycle number on line 1 and hold the button down to select the entire column. Then click on the "With Increment" at the bottom left of the screen. Your cycle numbers should now match the line numbers at the far left of the screen.

10. Fill in the calibration numbers for your standards at the far right of the table labeling the Timing Mix as "1" and labeling each sequentially so that Std E is "6".

11. At the upper left click on "File" and drag down to "Save as." Save the file as C:\Alcohol Data\VFLMMDDYYYY\VFLMMDDYYYY.seq. Exit by clicking on the inside "X" at the upper right.

12. Go to ChromPerfect Data Acquisition (black icon). The status screen should be up (blue top half), if not click on the status tab at the right of the screen.

13. Click on the "Control" icon (grey box/red button). The instrument control window should appear.

14. Click on the download button. The acquisition panel should be displayed (if not, click the appropriate tab.)

15. Make sure there is a check mark in the box labeled "Use Sequence File." Click on the down arrow and select the sequence file you just saved.

16. Click on "OK" at the bottom of the window.

17. At this point the display in the acquisition panel should have the sample name, method file name and raw file name from your first line of the sequence. The status column should display "ready."

18. The system is now waiting for a signal to be received from the Tekmar HT3.

19. Start the run by starting the Tekmar. (The Tekmar can be started before making and downloading the sequence if it can be completed before the Tekmar injects the first sample.)

Appendix B

Teledyne-Tekmar HT3 Autosampler Method Setup

1. Constant Heat Time: ON
2. G.C. Cycle Time: 16 min
3. Valve Oven Temp: 70°C
4. Transfer Line Temp: 75°C
5. Standby Flow Rate: 80 mL/min
6. Platen/Sample Temp: 65°C
7. Platen Temp Equil Time: 1.00 min
8. Sample Equil Time: 24 min
9. Mixer: OFF
10. Pressurize: 7 PSIG
11. Pressurize Time: 2.00 min
12. Pressurize Equil Time: 0.20 min
13. Loop Fill Pressure: 5 PSIG
14. Loop Fill Time: 0.60 min
15. Inject Time: 0.50 min

Sequence Setup

1. Click on the button for “Schedule”
2. Select “Schedule Builder”
3. Select the current method
4. Enter the start and stop vial locations
5. Save the sequence as the current date (i.e. VFLMMDDYYYY)
6. Click “Step”
7. Click “Make Active”
8. Click “Start”

Appendix C

Parameter Settings for the 6890N Gas Chromatograph

The following settings are to be used for the 6890N Gas Chromatograph for the analysis of this method. Only the front detector is used in this method. Any change to the setting should be documented in the 6890N Maintenance Logbook.

1. OVEN
    a. Initial Oven Temperature: 40°C
    b. Initial Time: 1.00 minutes
    c. Rate 1: 5.00 deg/minute
    d. Final Temperature 1: 60°C
    e. Final Time: 0 minutes
    f. Rate 2: 40.00 deg/min
    g. Final Temperature 2: 170°C
    h. Final Time 0.25
2. FRONT INLET
    a. Mode: Split
    b. Temperature: 220°C
    c. Pressure: 19.9
    d. Split Ratio: 60
    e. Total Flow: 125
    f. Gas Saver: OFF
3. COLUMN 1
    a. Dim 30.0m 250u
    b. Pressure: 19.9
    c. Flow: 2
    d. Velocity: 41
    e. Mode: Constant Flow
4. FRONT DETECTOR
    a. Temperature: 250°C
    b. H2 Flow: 40
    c. Air Flow: 400
    d. Mode: Constant Makeup
    e. Mkup (He): 45
    f. Flame: ON